United States
Department of
Agriculture
Forest Service
General Technical
Report NE-64
1981

U. S. Forest Service American Chestnut Cooperators' Meeting

by H. Clay Smith

Abstract

On January 8 and 9, 1980, a 2-day meeting of the U.S. Forest Service American chestnut cooperators was held at Pipestem State Park, Pipestem, West Virginia. All Forest Service chestnut cooperators were represented as were a number of other chestnut researchers. Topics discussed included laboratory studies of *Endothia parasitica*, field studies of hypovirulence, virology-biochemistry, and miscellaneous. A total of 43 talks were given at this meeting. All speakers provided short abstracts of their talks; these abstracts are presented here.

The Author

H. CLAY SMITH is project leader of the 1103 TM Project at the Northeastern Forest Experiment Station's Timber and Watershed Laboratory, Parsons, West Virginia. He received a B.S. degree from West Virginia University and a M.S. degree in silviculture from Purdue University. He has been working for the U.S. Forest Service in timber management research since 1962.

Cover Photo Credit

West Virginia University Agricultural Experiment Station, Morgantown, W. Va.

Foreword

Since the death of the American chestnut, there has been concern, resignation, and now new hope. This new hope is hypovirulence--a phenomenon that was initially observed in Italy. Many European chestnut trees have recovered from the blight and European researchers believe hypovirulence, less virulent strains of the chestnut blight, is the best explanation for this recovery. In the United States, hypovirulence has potential for biological control of the chestnut blight, but we realize there are many problems to solve before hypovirulence or any other control method will unlock the mystery of the chestnut blight.

On January 8 and 9, 1980, a 2-day meeting of the U.S. Forest Service American chestnut cooperators was held at Pipestem State Park, Pipestem, West Virginia. All Forest Service chestnut cooperators were represented, including participants from West Virginia University, Concord College, Virginia Polytechnic Institute and State University, Duke University, University of Kentucky, Utah State University, and the Southeastern Forest Experiment Station. Researchers from the Connecticut Agricultural Experiment Station also attended. Forest Service personnel from the Northeastern Forest Experiment Station and Washington Office were present, as were members of the West Virginia Department of Agriculture and the USDA Southeastern Fruit and Tree Nut Lab. A total of 43 talks were given at this meeting. All speakers provided short abstracts of their talks; these abstracts are presented here.

The purpose of the meeting was to provide an opportunity for the cooperators and other researchers to report the status of their cooperative research on American chestnut. The meeting allowed for an exchange of ideas among those in attendance; no doubt future research

will be improved by thoughts and ideas exchanged at the meeting. The program was divided into four sessions--

Laboratory cultural studies in *Endothia parasitica*;
Field studies (hypovirulence);
Virology-biochemistry;
Miscellaneous

After each session, there was an informal discussion among the participants.

At the final session, future American chestnut research needs were discussed, with special emphasis on how the hypovirulent phenomenon works. There was need for more quantitative research data to support research statements, and a need to establish more specific study objectives and priorities. Researchers also suggested a need to evaluate the effects of hypovirulent isolates on some selected hybrid tree species that were planted by Jesse Diller in the eastern United States between 1947 and 1955. Also a terminology guide for chestnut researchers was suggested.

Hypovirulence may not be a complete solution to the American chestnut problem, and it is doubtful that any one single system such as hypovirulence is going to provide the solution to the blight problem. In recent years, we believe, chestnut researchers in the United States have learned more about hypovirulence than in all the previous years combined. Cooperators and researchers were urged to continue their efforts. The American chestnut blight problem could not be solved in a given number of years even with unlimited funding. Presently lack of knowledge is more of a problem than lack of funds. However, funds and support are necessary to gain knowledge and to continue the research effort. The Forest Service was urged to continue to provide funding for this research program.

Presently most mature American chestnut trees within its natural range are dead. However, the species survives in nature through sprouts that develop from living roots. The American chestnut was a major tree species throughout the eastern forests until the blight, *Endothia parasitica*, eliminated it. With current emphasis and increasing concern, based on the European situation, a breakthrough is possible. Through cooperative efforts and free exchange of ideas as exhibited at this meeting, researchers hope to solve the mystery of the chestnut blight and allow the American chestnut to regain its prominence in the eastern hardwood forests.

--H. CLAY SMITH

Contents

Forest Service American Chestnut Cooperative Research Program

SMITH, H. Clay
USDA Forest Service
Timber and Watershed Laboratory
Parsons, West Virginia 26287

Though the economic and ecological impacts are impossible to assess, there is little doubt that the single greatest plant tragedy to occur in this country was the loss of the American chestnut tree. Efforts to control chestnut blight and reestablish American chestnut trees have been futile. Recently there has been a discovery in Europe that may eventually provide the basis to successfully reintroduce American chestnut trees in this country: Certain less pathogenic (hypovirulent) strains of the chestnut blight were reported to inhibit growth of more virulent strains of the blight, and many of the European chestnut trees have recovered from the blight.

In 1978, the U.S. Forest Service, through the efforts of Senator Robert C. Byrd and his associates, provided funding for American chestnut research. To date, approximately $300,000 has been obligated by the U.S. Forest Service for this research program. Because of the European situation, the U.S. Forest Service decided to select cooperators to evaluate the hypovirulence phenomenon as a possible biological control for the chestnut blight. Presently the Forest Service has seven cooperators conducting 11 studies. These cooperators and the major responsible researchers include:

Concord College - Department of Physical Sciences
John Elkins and Bruce Given (West Virginia Department of Agriculture)

Duke University - Department of Forest Pathology
William Stambaugh and Bruce Nash

University of Kentucky - Department of Plant Pathology
Louis Shain, Gerald Nordin, and John Russin

Virginia Polytechnic Institute and State University - Department of Plant Pathology
Gary Griffin, Fred Hebard, and John Weidhaas, Jr.

Utah State University - Department of Biology
Neal Van Alfen, James Bowman, and John Simmons

West Virginia University - Department of Plant Pathology
William MacDonald, Dale Hindal, and Walt Kaczmarczyk

Southeastern Forest Experiment Station
George Kuhlman

The cooperative research effort involves a variety of research interests in hypovirulence, as indicated in the accompanying abstracts. Nutritional and vegetative compatibility studies of virulent and hypovirulent isolates of *Endothia parasitica* are being evaluated in laboratory and field tests. The transmission and survival of chestnut blight and the dissemination and natural occurrence of chestnut blight on American chestnut trees and other hardwood tree species are also being evaluated. Forest Service cooperators are evaluating biochemical characteristics of the chestnut blight and learning more about the mechanism of hypovirulence. Also, a number of other researchers have been involved in this Forest Service cooperative research program by providing re-

views of study proposals and suggestions for future research. They include Richard Jaynes, John Elliston, and Sandra Anagnostakis of the Connecticut Agricultural Experiment Station; Dave Houston, Fred Berry, and Robert Phares, Northeastern Forest Experiment Station; George Kuhlman and Sam Gingrich, Southeastern Forest Experiment Station; and Ed Wicker and Gerald Anderson, U.S. Forest Service, Washington Office.

Cooperators have initiated field studies on public lands including the Jefferson, Monongahela, Natahala, Pisgah, George Washington, and Daniel Boone National Forests. Also study areas are established at the Coweeta Hydrological Lab in Franklin, North Carolina, Bent Creek Experimental Forest in western North Carolina, and Fernow Experimental Forest, Parsons, West Virginia, and on private lands in West Virginia, Virginia, North Carolina, and Kentucky.

The following abstracts from West Virginia University, Duke University, Concord College, Virginia Polytechnic Institute, Utah State University, University of Kentucky, and Southeastern Forest Experiment Station report current cooperative research being funded by the U.S. Forest Service. Much of this research is just beginning and plans are to have another cooperators' meeting in 3 or 4 years to assess the status of the research and provide future direction for the program. Since results and data trends will be more definite, papers will be presented and published at that meeting. In the meantime, we will all keep abreast of developments in the various American chestnut research programs throughout the country.

A. Laboratory Cultural Studies of *Endothia parasitica*

a. Nutritional Studies

Utilization of Hamamelitannin by *Endothia parasitica*

ELKINS, John R., GRAHAM, Patricia, and PATE, Wesley
Division of Natural Sciences
Concord College
Athens, West Virginia 24712

Endothia parasitica utilized hamamelitannin, a diester of gallic acid and hamamelose (2-C-hydroxymethyl-ribose), from the aqueous extracts from the bark of American chestnut and from a minimal medium amended with either a purified tannin fraction from American chestnut bark or purified hamamelitannin as the only carbon source. The rate of utilization of purified hamamelitannin by each of four strains of *Endothia parasitica*, including a native hypovirulent strain, was similar as monitored by high pressure liquid chromatography; so all four rates were averaged together. Rapid utilization of hamamelitannin, and consequent build-up of gallic acid, began 1 day after inoculation and was essentially complete 4 days after inoculation. Such a rapid rate of utilization of hamamelitannin could be instrumental in the colonization of the American chestnut since Bazzigher has reported that inoculations of the susceptible European chestnuts made 3 days after wounding do not result in growth of the fungus.

Nutritional Comparisons among Normal and Hypovirulent Isolates of *Endothia parasitica*

HINDAL, Dale F.
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

Nutritional studies were conducted to compare growth (production of mycelium in liquid media) among two normal, two white hypovirulent, and one pigmented hypovirulent isolate of *Endothia para-*

sitica. Among 12 nitrogen sources tested, casamino acids, asparagine, arginine, histidine, and ammonium tartrate supported best mycelium production when glucose was used as the carbon source. These nitrogen sources were then tested in combination with four additional carbon sources: fructose, sucrose, cellobiose and soluble starch. Generally, the two white hypovirulent isolates produced the most mycelium, the two normal isolates intermediate amounts, and the pigmented hypovirulent isolate the least mycelium. However, regardless of the nitrogen source, soluble starch did not support as much mycelium production as the other carbon sources, and the mycelium production by the pigmented hypovirulent isolate was more drastically reduced on the soluble starch media than other isolates. The white hypovirulent isolates produced abundant mycelium on all carbon sources with histidine as the nitrogen source, whereas the normal and pigmented hypovirulent isolates produced less mycelium on a fructose or sucrose medium when histidine was the nitrogen source. These studies are continuing, but the data indicate there are striking differences in nutritional requirements for optimum mycelium production among these isolates of *Endothia parasitica*.

b. Vegetative Compatibility

Vegetative and Sexual Compatibility in *Endothia parasitica*

ANAGNOSTAKIS, Sandra L.
Connecticut Agricultural Experiment Station
P.O. Box 1106
New Haven, Connecticut 06504

Sexual incompatibility is homogenic. So far 108 strains have been crossed with two mating type testers and only one locus with two alleles has been found. Occasional evidence for homothallism is seen. The mating type distribution so far examined has yielded the following:

USA:	a	33
	A	27
Italy:	a	3
	A	2
selfs		2
Greece:	a	7
China:	a	2
	A	2

The mating type gene does not function as a vegetative incompatibility gene as it does in *Neurospora crassa*. Vegetative incompatibility is heterogenic, and so far 77 vegetative compatibility groups have been found. Tentative genotypes, based on a system of seven loci with two alleles, have been assigned for six of the vegetative compatibility groups.

Microscopic Study of the Morphology, Growth Patterns, and Interactions of *Endothia parasitica*

NORTHUP, Theresa
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

A defined medium has been developed that allows microscopic observation of the intra- and inter-hyphal fusions in *Endothia parasitica*. Hypovirulent and virulent isolates were grown singly or paired on this medium for 3 days, then prepared for light and scanning electron microscopic examination. After 3 days, the mycelial growth patterns of virulent and hypovirulent isolates appear identical microscopically. Intra-hyphal fusions were numerous in the four isolates examined. Fusion between paired compatible and incompatible isolates was not observed in the hyphal tip area.

c. Perithecial Formation

Controlled Crosses of *Endothia parasitica* in the Laboratory

ANAGNOSTAKIS, Sandra L.
Connecticut Agricultural Experiment Station
P.O. Box 1106
New Haven, Connecticut 06504

The method published in Mycologia (*71*, 213-215, 1979) still proves to be reliable. *Endothia* salt solution is now substituted for water for a slight improvement (it supplies nitrogen). The best wood for use in this technique is American chestnut, with red maple a close second. Yellow birch and white oak were minimally successful. The following species failed to support perithecial development: black oak, post oak, black cherry, and white pine.

Artificial Introduction of Virulent and Hypovirulent Strains of *Endothia parasitica* Using Large Scratch Wounds

MACDONALD, William L.
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

One virulent and 11 hypovirulent isolates of *Endothia parasitica* were introduced into scratch wounds made on the bark of healthy American chestnut stems growing in two cut-over areas near Parsons and Bartow, West Virginia. Twenty-five-cm scratch wounds, encircling half the circumference of the tree, were made at ground level and at 1.5 m and 3.0 m off the ground. Replicate stems were scratched and inoculated with one of the hypovirulent isolates. A second group of healthy trees (40 to 90) was left unwounded within the plot. Other check plots included trees that were scratched and inoculated with water and agar, scratched and inoculated with virulent inoculum, and a plot where trees were not scratched. An attempt was made to reduce virulent inoculum in all study plots by removing infected trees.

The goal of this study is to follow the course of future infections on both the scratched trees and various check trees. To do this, isolations will be made from new infections that arise on trees within the various treatment plots. By introducing massive amounts of hypovirulent inoculum, it is hoped that evidence for the establishment of hypovirulent strains can be acquired.

Worldwide Distribution of Vegetative Compatibility Groups

ANAGNOSTAKIS, Sandra L.
Connecticut Agricultural Experiment Station
Box 1106
New Haven, Connecticut 06504

There are no vegetative compatibility types in Europe that have not been found in the Connecticut Agricultural Experiment Station sample. However, four isolates from China are unlike each other and not like any we have at Connecticut. Greece, with 14 isolates examined from the only region reporting chestnut blight, has yielded only one vegetative compatibility type.

Connecticut	67 total vegetative compatibility groups
all USA	77 total vegetative compatibility groups
Italy	9 vegetative compatibility groups among 37 isolates
France	20 vegetative compatibility groups among 34 isolates
Greece	1 vegetative compatibility group among 14 isolates

B. Field Studies of Hypovirulence

a. Effect of Hypovirulent Strains on Virulent Cankers

Two-year Control Results in Artificially Established Virulent Cankers with Compatible and Incompatible Hypovirulent Strains at Three Locations in West Virginia

DOUBLE, Mark L.
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

Eight virulent cankers were artificially established on each of 24 American chestnut trees in three areas of West Virginia. The inoculations were made 6 to 8 inches apart on each tree in July 1978, using eight different virulent strains of *Endothia parasitica*. The compatibility type of each strain was predetermined in the laboratory using the procedure of Anagnostakis. Cankers were challenged after 1 month, with either individual or mixed hypovirulent isolates. The challenge was accomplished by punching 0.5 cm holes every 1 to 2 cm around the entire margin of the canker and then introducing the appropriate hypovirulent inoculum. Water agar was used in place of hypovirulent inoculum in control (check) trees. The length and width of each canker were measured at the time of the challenge and then 3, 8, and 14 months after inoculation.

Laboratory tests, pairing virulent and hypovirulent strains of *Endothia parasitica* on an amended potato-dextrose agar, were used to determine compatible and incompatible virulent-hypovirulent combinations. The results of the laboratory and field tests were comparable. In every instance, when a hypovirulent isolate or mixture of isolates converted a virulent isolate in the laboratory, it controlled the same isolate in the field test. There were certain virulent isolates, however, that were controlled in the field but were not converted in laboratory tests. Thus, it appears that the physical make-up of the tree affords a greater opportunity for conversion to occur.

Efficiency of Conversion of Virulent Strains to Hypovirulent in the Host and in the Laboratory

ANAGNOSTAKIS, Sandra L.
Connecticut Agricultural Experiment Station
Box 1106
New Haven, Connecticut 06504

Efficiency of conversion is low on plain agar media unless strains paired are in the same, or closely related, vegetative compatibility groups. Efficiency is much better on the surface of cellophane over agar media. A single hypovirulent strain and 50 different normal strains were paired on cellophane and in the host; 25 of the 50 vegetative compatibility types were converted at least once on cellophane, compared to 37 of 50 in trees.

Mixtures of Hypovirulent Strains of *Endothia parasitica*: Their Pathogenicity and Ability to Control Cankers on American Chestnut

JAYNES, Richard A. and ELLISTON, John E.
Connecticut Agricultural Experiment Station
Box 1106
New Haven, Connecticut 06504

Pathogenicities of normal and hypovirulent strains of *Endothia parasitica* are highly variable and strain dependent. Pathogenicity of hypovirulent strains decreases dramatically when two or more are mixed. This is probably due to superinfection with different hypoviru-

lence factors and the cell death which results from the interaction of hyphae from members of different vegetative compatibility groups. Mixtures of different hypovirulent mycelia effectively overcame vegetative incompatibility and rapidly arrested canker development. However, mixtures tested to date may be too debilitated to survive and disperse themselves in the wild.

Effectiveness of Slurry Treatments in Controlling Individual *Endothia parasitica* Cankers on American Chestnut

MACDONALD, William L. and DOUBLE, Mark L.
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

Four different slurries of hypovirulent strains were used to treat virulent *Endothia parasitica* cankers on American chestnut growing at three locations in West Virginia. The slurries included: a mixture of debilitated white strains (B-type); a mixture of Italian strains of intermediate pathogenicity; a pigmented but debilitated mixture of strains (Jr-type); and, a general slurry containing a combination of all strains (General). New cankers were treated at monthly intervals as they developed from May to November by punching 0.5 cm holes to the cambium at 1 cm intervals around the canker margin.

Many of the trees in all treatments have produced callus tissue in response to treatment. However, the number of new infections is discouraging as many trees that responded well to treatment have been killed by subsequent infections. In a preliminary analysis of variance, designed to compare the effectiveness of the four slurries, the General and B-type slurries were significantly more effective in checking canker expansion. Both of these slurries contained the greatest number of debilitated strains. The majority of the data collected during the 3-year study must still be analyzed.

An Objective Method for Estimating Biocontrol of Hypovirulent and Virulent Cankers

HEBARD, Fred V., and GRIFFIN, Gary J.
Department of Plant Pathology and Physiology
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

ELKINS, John R.
Division of Natural Sciences
Concord College
Athens, West Virginia 24712

An objective method was devised for estimating biocontrol of cankers in hypovirulent plus virulent coinoculations. Canker lengths were determined biweekly during summer and early fall for virulent and virulent plus hypovirulent cankers located on the same tree. A hypovirulent plus virulent canker was deemed biocontrolled when its growth rate was significantly less ($p<0.05$) than the growth rate of its companion virulent canker.

This method was used in one experiment involving 13 hypovirulent plus virulent combinations replicated four times. Inoculations for the experiment were made from June 15 to June 19, 1978. Sixteen virulent plus hypovirulent cankers showed biocontrol in 1978. In 1979, four additional cankers showed biocontrol. On comparing growth rates for the two growing seasons, it was apparent that the additional biocontrol occurred after the first growing season. Of the hypovirulent plus virulent cankers showing biocontrol in 1978, 13 of 16 survived the 1979 growing season. Death of the stem where the cankers were located was due to natural virulent infections on basal

stem parts in two of three cases. In the third case, the hypovirulent plus virulent canker did not grow rapidly until its companion virulent canker had girdled the stem and killed its distal parts.

Among the surviving biocontrolled cankers, there were four cases of possible "reversal" of biocontrol. Definite judgment on whether biocontrol occurred in these experiments will depend on results of pathogenicity tests of multiple isolates from each biocontrolled canker.

Pathogenicity and Sporulation of Virulent and Hypovirulent Strains of *Endothia parasitica*

WILLEY, Ronald
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

Two virulent and seven hypovirulent strains of *Endothia parasitica* were screened for pathogenicity, asexual and sexual sporulation. Strains, representing collections from France, Italy and the United States, were inoculated into 2- to 10-year-old American chestnuts for evaluation of pathogenicity and sporulation. Over a 7-month period, virulent strains invaded host tissue extensively with 98 percent of the cankers showing evidence of asexual sporulation. Single conidial cultures obtained from virulent laboratory cultures and field cankers were morphologically similar to the parental strains. Cankers produced by Italian and American hypovirulent strains were from 10 to 85 percent smaller than those from virulent strains while one French hypovirulent strain was nonpathogenic. In contrast to virulent cankers, asexual sporulation was observed in only 64 percent of the hypovirulent cankers. Because fewer stromata were observed on hypovirulent cankers, pycnidial production may be correspondingly lower. Single conidial cultures from American hypovirulent strains resembled the parental strain. As many as three different morphological types were observed in single conidial cultures from Italian hypovirulent strains. Perithecia were present in 67 percent of the virulent cankers in the field, but were absent on all hypovirulent cankers. Laboratory crosses between sexually compatible virulent and hypovirulent strains resulted in perithecia only when conidia from hypovirulent isolates were applied to the virulent mycelium.

Pathogenicity, Growth, and Sporulation of Virulent and Hypovirulent Isolates of *Endothia parasitica* in the Southern Appalachians

KUHLMAN, E. George
USDA Forest Service
Southeastern Forest Experiment Station
Research Triangle Park, North Carolina 27709

Initial studies with hypovirulent isolates of *Endothia parasitica* in North Carolina suggested that three factors might limit the utility of this phenomenon for biological control in the Southern Appalachians. These factors are a slow rate of spread, short-term survival, and vegetative incompatibility among isolates.

Eleven months after inoculation, the canker width for six hypovirulent isolates of *Endothia parasitica* averaged 2.8 mm whereas the width for six virulent isolates was 63.2 mm. There was no apparent sporulation by hypovirulent cankers whereas 50 percent of the surface of virulent cankers had sporulation. How could pathogenicity and sporulation be improved? One possibility was to look for more susceptible host tissue. However, four hypovirulent isolates showed almost no virulence when injected

into small (less than 0.5-inch diameter) sprouts. In contrast, four virulent isolates caused cankers on 84 percent of the stems. In 1979, similar small sprouts were inoculated with hypovirulent or virulent isolates at three times during the growing season. Stem sections were inoculated at the same time to serve as a saprophytic check. The interim results of this experiment suggest limited invasion by the hypovirulent isolates in contrast to vigorous invasion and sporulation by virulent isolates.

Lab and Field Studies of Hypovirulent Strains: EP-60 from Michigan and EP-234 from Tennessee

ELLISTON, John E.
Connecticut Agricultural Experiment Station
Box 1106
New Haven, Connecticut 06504

EP-60 behaves as if it contains two factors conferring hypovirulence. One factor causes complete loss of pathogenicity, the other causes partial loss of pathogenicity. Each affects cultural characteristics differently. The effects of the more debilitating factor are dominant when the two factors are present together in the same strain. Isolates containing one, both, or neither of the factors were obtained by single spore isolation. The factors were transmitted separately and together by hyphal anastomosis from the original genetic background of the fungus into a genetically marked one and then returned to the original one. In both, each factor confers its distinct abnormalities in cultural characteristics and pathogenicity.

EP-234 behaves as if it contains a single factor conferring hypovirulence. This factor causes abnormalities in the fungus that resemble the abnormalities caused by the more debilitating factor in EP-60. This factor was transmitted to the marked genetic background where it has essentially identical effects on cultural characteristics and pathogenicity. The less debilitating factor from EP-60 and the factor from EP-234 were combined in the EP-60 genetic background by hyphal anastomosis. The effects of the factor from EP-234 were dominant in the product. These experiments illustrate the "simple" behavior of these particular factors from native American hypovirulent strains.

Periodic Inoculation of Virulent and Hypovirulent Strains of *Endothia parasitica* at 2-month Intervals

MACDONALD, William L.
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

The growth and sporulation of one virulent and seven hypovirulent isolates of *Endothia parasitica* are being evaluated following their periodic inoculation into American chestnut stems at Bartow, West Virginia. Initial inoculations were made in August 1979, with subsequent inoculations at 2-month intervals. A hole made with an 0.5 cm arch punch was used as the inoculation site. To date, measurable growth has occurred with all virulent and hypovirulent isolates. In contrast to the other isolates, an Italian hypovirulent isolate, EP-49, grew better during the October-to-December interval than during the August-to-October period. None of the cankers had sporulated by December 1979.

Lab and Field Studies of Hypovirulent Strains: Isolates from Italy

ELLISTON, John E.
Connecticut Agricultural Experiment Station
Box 1106
New Haven, Connecticut 06504

Bark samples were taken from numerous abnormal cankers on *Castanea sativa* in northern, central, and southern Italy in August 1978. The strain composition in many of these samples is complex. Abnormal "white" strains were obtained from many of these samples, often in combination with normal strains. The white strains differed widely in pathogenicity, in some cases even when obtained from the same bark sample. Single spore isolation of several of the more debilitated white strains yielded mixtures of white and heavily pigmented abnormal strains as well as normal strains. The abnormal strains obtained differed widely in pathogenicity and double-strand RNA was detected in all of the abnormal strains tested but not in the normal strains. Clearly, these strains differ greatly from American hypovirulent strains that have been studied in detail. Their complex behavior has not been explained adequately.

b. Histopathology

Histopathological Events During the Development of Cankers on Chestnut Species Incited by Virulent (V) and Hypovirulent (H) Strains of *Endothia parasitica*

HEBARD, Fred V., and GRIFFIN, Gary J.
Department of Plant Pathology and Physiology
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

ELKINS, John R.
Division of Natural Sciences
Concord College
Athens, West Virginia 24712

The time-course of histopathological events following artificial inoculation with *Endothia parasitica* was studied in highly resistant *Castanea mollissima* "Nanking" and in a fully susceptible and a partially resistant *Castanea dentata*. In all three types, a necrotic region, ca. 0.5 cm larger than the inoculation wound, formed when a virulent or hypovirulent strain of *Endothia parasitica* was present. Ten days after inoculation, this lesion, or wound of the uninoculated check, was surrounded by a lignified zone. For all species and treatments, wound periderm formation had commenced by 14 days at the nonnecrotic border of the lignified zone. At 10 days, the necrotic regions of all host-parasite combinations were infiltrated with individual hyphae; mycelium build-up had commenced by 14 days in the necrotic region of the compatible combination (e.g. virulent and fully susceptible) but was delayed in incompatible combinations (e.g. hypovirulent and highly resistant). By 18 days, an advancing mycelial fan had penetrated the lignified zone and developing wound periderm in the compatible combination; this was delayed in less compatible combinations (e.g. hypovirulent and fully susceptible and virulent or hypovirulent and partially resistant) and did not occur in incompatible combinations. The results suggest that the crucial component of a compatible reaction is the ability of *Endothia parasitica* to obtain nutrition from, and not be inhibited by, dying tissue of *Castanea* species.

c. Transmission and Survival of Hypovirulent and Virulent Strains

Distribution and Frequency of Vegetative Compatibility Types of Virulent *Endothia parasitica* Strains near Parsons, West Virginia

DOUBLE, Mark L.
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

The distribution and frequency of vegetative compatibility types of virulent isolates to *Endothia parasitica* were examined over a 3-year period from 1977 to 1979. Isolates were obtained from cankers on American chestnut stems found in two areas near Parsons, West Virginia. After pure cultures of *Endothia parasitica* were obtained from each canker, the compatibility type was determined by pairing mycelial plugs on amended potato dextrose agar. For the 3 years combined, a total of 449 isolates have been examined and 324 (72 percent) have been classified into 16 vegetative compatibility groups. In 1977, 33 isolates were classified in 9 vegetative compatibility groups; in 1978, 172 isolates were classified in 13 vegetative compatibility groups; and 244 isolates were classified in 14 vegetative compatibility groups in 1979. As the total number of cankers increased, so did the number of vegetative compatibility groups. However, the six major vegetative compatibility groups from 1977 remained the same through 1979. Since 1977, 125 isolates (28 percent) have been found that are vegetatively incompatible with the 16 vegetative compatibility groups. These isolates are currently being paired with each other to determine if additional vegetative compatibility groups exist.

Saprophytic Survival, Growth and Sporulation of Virulent and Hypovirulent Isolates of *Endothia parasitica* on Red Maple, Red Oak, and American Chestnut

BAIRD, Richard
Department of Plant Sciences
West Virginia University
Morgantown, West Virginia 26506

To study the survival, growth and sporulation of one virulent and six hypovirulent isolates of *Endothia parasitica*, 10- to 15-year-old girdled stems of American chestnut, red oak, and red maple were artificially inoculated in August, 1979. Stems of red oak and red maple were killed by girdling with an axe; chestnut stems were girdled by inoculation with a virulent isolate of *Endothia parasitica*. The chestnut stems had not died after 5 months, therefore, the data are not yet available for this host.

Data are summarized for red maple and red oak 4 months after girdling. Survival of *Endothia parasitica* in red maple, as determined by culture of bark plugs from the inoculation point, was 85 percent for the virulent isolate and ranged from 5 to 58 percent for the hypovirulent isolates. In red oak, the virulent isolate was recovered 75 percent of the time from the inoculation site while the hypovirulent isolates were recovered from 25 to 100 percent of the time. The virulent isolate was recovered 69 percent and 63 percent of the time at 1 cm and 3 cm beyond the inoculation site, while hypovirulent isolates were recovered at 1 cm from the inoculation site from 19 to 63 percent of the time and at 3 cm from the inoculation site from 0 to 63 percent of the time.

The Incidence of *Endothia parasitica* on Post and Scarlet Oaks in the Duke Forest

BONSTEDT, Steve M.
Duke University
School of Forestry and Environmental Science
Durham, North Carolina 27706

A survey was made of the Korstian and Durham Divisions of the Duke Forest to determine the incidence of *Endothia parasitica* on post oak (*Quercus stellata*) and scarlet oak (*Quercus coccinea*). A 35-compartment sample of the Durham Division showed 48 percent of the scarlet oaks and 33.3 percent of the post oaks to be infected, while an 18-

compartment sample of the Korstian Division, 77.7 percent of the scarlet oaks and 37.5 percent of the post oaks were infected. Infected trees ranged widely in dbh (3.0 - 17.9 in). Butt swell was noted on less than half of the infected scarlet oaks, while only stem cankers occurred on the post oaks.

Etiology of *Endothia parasitica* on Scarlet Oak

STAMBAUGH, William J.
Duke University
School of Forestry and Environmental Science
Durham, North Carolina 27706

In August 1963, a swollen butt condition of scarlet oak with multiple cankers was first noted on the James River and Warm Springs Ranger Districts of the George Washington National Forest. Swollen butt taper showed a diameter ratio of 2.2 basally to a height of 2.65 feet, as compared to 1.3 to 0.65 feet for healthy trees. Isolations from bright orange fans associated with inner bark necrosis yielded an *Endothia*-like fungus, tentatively identified as *Endothia parasitica*.

The disease has since been observed on all divisions of the Duke Forest, Durham, North Carolina, and elsewhere in the state. Consistent with these observations were pycnidia with rod-shaped conidiospores matching those of *Endothia parasitica*, which occurred sparsely in the bark fissures of all affected trees examined; excluding a recent find, perithecia were lacking. Known isolates of the fungus from chestnut duplicated the disease in scarlet oak 16 months after artificial inoculation of basally-wounded young forest trees with either mycelial or conidial inoculum. The number of infections (22 percent) thus obtained may be limited by tree age, as suggested by a subsequent ring-dating study of naturally infected trees 42 to 68 years old and averaging 13.7 cankers per tree (range 4 to 27 cankers), of which 76 percent were not infected until after age 25. Comparison of those trees with healthy individuals did not show significant growth reduction attributable to infection; however, the swollen butt condition, because of stain and bark inclusions, makes cull of that portion of the first log.

d. Natural Occurrences of Virulent and Hypovirulent Strains in the United States

Abnormal Strains of *Endothia parasitica* Associated with Large Surviving American Chestnut Trees

JAYNES, Richard A.
Connecticut Agricultural Experiment Station
P.O. Box 1106
New Haven, Connecticut 06504

Endothia parasitica have been isolated from 20 large (mostly 12-inch dbh or more) surviving American chestnut trees in seven states. Abnormal isolates have been selected from these trees. Many are hypovirulent in the sense that they are less pathogenic than normal strains when tested on excised and *in situ* American chestnut stems, and they contain dsRNA. The evidence leads us to believe that many of these surviving trees are not genetically resistant but owe their survival to being infected with abnormal (hypovirulent) strains of *Endothia parasitica*.

Proportion of the *Endothia parasitica* Biomass that is Hypovirulent in Two Surviving American Chestnut Trees

GRIFFIN, Gary J., and HEBARD, Fred V.
Department of Plant Pathology and Physiology
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

ELKINS, John R., and GALLUZZI, Kate
Division of Natural Sciences
Concord College
Athens, West Virginia 24712

Tissue samples from the lateral or inner radial margins of cankers on two surviving American chestnut trees were plated on acidified potato-dextrose agar. *Endothia parasitica* isolates growing from these infected tissues were tested for pathogenicity on American chestnut stump sprouts in the Jefferson National Forest. Of approximately 100 isolates obtained from all portions of an American chestnut tree growing near Centerville, Virginia, about one-third were hypovirulent (weakly pathogenic), slightly more than half were virulent, and the remainder were intermediate. Hypovirulent isolates produced small cankers (5 cm or less in length after 4 months), and only superficial infection of the bark. All of about 28 isolates obtained from an American chestnut tree growing near Summers, West Virginia, were virulent.

Implications of Chestnut Blight Incidence in Recently Clearcut and Mature Forests for Biological Control of Blight with Hypovirulent Strains of *Endothia parasitica*

HEBARD, Fred V. and GRIFFIN, Gary J.
Department of Plant Pathology and Physiology
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061

ELKINS, John R.
Division of Natural Sciences
Concord College
Athens, West Virginia 24712

In recently clearcut sites, the incidence of chestnut blight increased from about 20 percent, the level in mature forest areas, to 100 percent between 4 and 9 years after clearcutting. During this period, the increase was linear with time. Disease progress was of the compound interest type, indicating that inoculum for the epidemics was generated in the clearcut sites. The disease-progress curve could be modeled when provision was made for an increase in canker area with an increase in tree diameter. This provision explained the 4-year delay in the start of the epidemics: sporulating area, and thus inoculum production, was dependent on canker area. The mean diameter at breast height of chestnut sprouts greater than 0.8 cm diameter was smaller at mature forest sites than that at 6-year-old or older clearcut sites, presumably because the sprouts at mature forest sites were shaded. Thus, sprout size alone may explain the low incidence of chestnut blight at mature forest sites.

However, there are additional factors that may have impeded epiphytotics on mature forest sites and favored them in clearcuts. Larger (greater than 3 cm diameter) stem parts at mature forest sites, but not clearcut sites, had dead outer bark (rhytidome), which decreased the number of stromata per unit canker area. On trees with rhytidome, sporulation occurred only in cracks or gaps in the rhytidome which reached the inner bark. Rhytidome also may have decreased the frequency of infection courts (wounds). There were more sprouts per clump in clearcuts and this may have helped epiphytotics start in clearcuts by favoring the spread of the pathogen

within sprout clumps, and thus the build-up of inoculum.

The sporulating area per 20- x 20-m plot was 500 cm^2 when incidence increased above the starting level in clearcuts. This suggests that this much sporulating area of hypovirulent strains of *Endothia parasitica* may be needed to start an outbreak of hypovirulence. It will be necessary to control virulent strains in released chestnut sprouts. Thus, it appears that it would be better to conduct hypovirulence deployment experiments at clearcut sites than at mature forest sites. The rapid rate of disease progress indicates that it would be necessary to treat young (ca. 4-year-old) clearcuts to avoid an outbreak of virulent strains of *Endothia parasitica*.

Before effective strategies for deploying hypovirulence can be devised and implemented, it will be necessary to know the canker growth and sporulation characteristics of average hypovirulent isolates from Italy, and the distribution of vegetative compatibility groups there. Also, it will be helpful to know the role of host resistance and the weather in virulent canker growth, sporulation, and disease progress in Europe.

Endothia Canker Survey of Chestnut and Oaks in the Mountain Counties of North Carolina

NASH, Bruce
Duke University
School of Forestry and Environmental Studies
Durham, North Carolina 27706

Sample plots (6 for each of 21 North Carolina counties) were randomly selected using the grid system on county maps. On location, each plot was divided into five scatter plots, according to U.S. Forest Service survey methods. Within each scatter plot, data were obtained pertaining to species composition, tree measurements, and characterization of all *Endothia* infections; appropriate symptom and sign material was collected for laboratory isolations. To supplement the *Endothia* isolates thus obtained, additional symptomatic trees were sampled at approximately 10-mile intervals along the Blue Ridge Parkway in North Carolina. The data will be computerized, and isolates (267 to date) will be characterized as to vegetative compatibility group and virulence.

Preliminary results show that 72 of the 126 plots contained scarlet oak, of which 8.4 percent were infected with *Endothia parasitica*. American chestnut was present in 36 of the 126 plots with approximately 28.5 percent infection. A total of 407 scarlet oaks and 789 chestnut stems or sprout clumps were examined in this mountain sample.

e. Dissemination by Vectors

Dissemination of *Endothia parasitica* by Birds and Small Mammals

DEPALMA, Nancy
Connecticut Agricultural Experiment Station
P.O. Box 1106
New Haven, Connecticut 06504

Birds and mammals travel up and down trees in their search for food and shelter. Mammals also spend time in the soil and litter beneath trees. *Endothia parasitica* has been recovered from both birds and mammals from chestnut plots in Connecticut. While it is improbable that birds and mammals are major vectoring mechanisms, they do come in contact with the spores and may play a role in spore movement, especially within small areas.

Insects as Potential Vectors of Hypovirulent *Endothia parasitica* Strains

RUSSIN, John S.
University of Kentucky
Department of Plant Pathology
Lexington, Kentucky 40506

A survey of insects that may serve as potential vectors of hypovirulent *Endothia parasitica* strains was conducted from April to October 1979. Insects were sampled from bark of healthy, infected and dead American chestnut (*Castanea dentata*) stems and from additional tree species frequently found in association with chestnut. The major sampling method used involved tanglefoot-coated fiberglass screening affixed to diseased or healthy bark of the host tree. These screen traps were removed and replaced biweekly. Results indicate that the habituating insect fauna of American chestnut is mostly confined to two orders, Coleoptera and Diptera. The major coleopteran families include Eucnemidae, Scolytidae, Bostrichidae, and Staphylinidae, while Sciaridae, Phoridae, and Dolichopodidae were the major dipteran families. Differences were observed in species diversity and total number of insects collected between the types of host; dead infected stems attracted the most and healthy stems attracted the least numbers of insects and insect species. Other hardwood species similarly sampled show a somewhat different insect fauna.

Potential Insect Vectors of *Endothia parasitica* Associated with Chinese Chestnut and Post and Scarlet Oaks

SCHROEDER, Robert A.
Duke University
School of Forestry and Environmental Studies
Durham, North Carolina 27706

Insects were collected periodically during the fall and winter from traps installed on healthy and cankered bark of five trees in each of the above species on the Duke Forest. Of 566 insects trapped, ants (Hymenoptera; Formicidae) and fungal gnats (Diptera; Mycetophilidae) made up 90 and 6 percent of the total. Carpenter ants (*Camponotus* sp.) were collected in greater abundance (8x) from healthy bark than from cankered bark of scarlet oak. Fungal gnats may be more suspect in transmission potential because of their more constant association with all tree species investigated.

A post oak canker caged indoors for 43 days yielded 66 emergent insects, of which 85 percent were ants in the genus *Crematogaster*. The ants in particular, other than *Camponotus* sp., merit further study.

Gall Wasp: Status and Future Studies

PAYNE, Jerry A.
Southeastern Fruit and Tree Nut Laboratory
Byron, Georgia 31008

The oriental chestnut gall wasp is presently confined to Chinese chestnut trees in central Georgia. Rate of movement is approximately 15 miles per year. It is known to attack American chestnuts; however, present distribution of gall wasp is outside the range of American chestnut. Information was presented on possible approaches to control the gall wasp.

C. Virology--Biochemistry

Properties of dsRNA and Particulate Components Associated with Hypovirulence

DODDS, J. Allan
Connecticut Agricultural Experiment Station
P.O. Box 1106
New Haven, Connecticut 06504

Two distinct patterns of multiple double strand-RNA (dsRNA) components are now recognized; one typical of most European hypovirulent strains and the other typical of North American hypovirulent strains. The revised molecular weights of the prominent dsRNA's of both types are between 4.0 and 7.0 x 10^6, unusually high for a fungal virus genome. The number and quantity of dsRNA components havebeen associated with levels of hypovirulence in isolates obtained from single conidia of a North American strain (EP-60). The quality of dsRNA varies unpredictably in the strain with the most complex dsRNA pattern (EP-113). The properties of nucleic acids and particulate components in extracts of *Endothia parasitica* before and after conversion by a hypovirulent strain (EP-113) have been compared. One result of infection is the presence of large amounts of two membranous fractions separable by density-gradient centrifugation and not present in the unconverted strain. Only one of these fractions has dsRNA associated with it; a fraction of this kind has been detected in several European hypovirulent strains. The particles in this fraction are club-shaped and do not resemble any well-characterized fungal virus.

Procedures Used to Extract Double-strand RNA

NEBIOLO, Mark
West Virginia University
Department of Plant Sciences
Morgantown, West Virginia 26506

A general procedure for isolating double-stranded RNA (dsRNA) was described. The procedure is based on conventional techniques employing CF-11 chromatography and polyacrylamide gel electrophoresis. The procedure was adapted for routine use in screening for dsRNA in small quantities of *Endothia parasitica*. A method based on the differential solubility of nucleic acids in lithium chloride was judged to be useful primarily for isolating dsRNA from batch samples.

All virulent strains isolated in the field lacked dsRNA, but many suspected hypovirulent strains contained dsRNA, as did all isolates obtained from Italian and French healing cankers. The dsRNA gel profiles exhibited one, two, and three major bands on 5 percent gels. All European strains tested appear to have a single common band. Current work is on determining the dsRNA status of isolates obtained from hypovirulent-like cankers on American chestnut growing in Michigan.

Purification of Virus-like Particles from a Hypovirulent Strain of *Endothia parasitica*, EP-43

CHMELO, Richard
West Virginia University
Department of Plant Sciences
Morgantown, West Virginia 26506

Virus-like particles were purified from a hypovirulent strain of *Endothia parasitica*, EP-43. The method used is a modified version of Dodds' procedure; 50 to 100 grams of fungal mycelia are homogenized, subjected to polyethylene glycol precipitation, differential centrifugation, and sucrose density gradient centrifugation. One ml fractions were collected and read in a spectrophotometer at 254, 260, and 280 nm. Fractions with the highest optical density values were dialyzed against four changes of buffer. The dialyzate contained the purified virus-like particles. Steps used during the procedure to quantify the amount of dsRNA present included: SDS-phenol extraction, ethanol precipitation, and Whatman CF-11 cellulose column chromatography. Purified virus-like particles were placed on carbon-coated grids, stained with phosphotungstic acid, and examined under the electron microscope.

The virus-like particles were found to be pleomorphic, but averaged 90 nm in diameter. After treatment with chloroform, no virus-like particles were seen.

Attempts to Produce Antibodies to Synthetic Double-strand RNA Poly Inosine: Cytosine

STOCKHAUSEN, Anne
West Virginia University
Department of Plant Science
Morgantown, West Virginia 26506

Attempts were made to produce antibodies to synthetic double-strand RNA (dsRNA) as a technique for rapid field assay for dsRNA in *Endothia parasitica* and to correlate the amounts and types of dsRNA with hypovirulence. A weakly synthetic poly I:C (Inosine:Cytosine) injection was administered intramuscularly to 4.5-pound New Zealand white rabbits for 3 weeks, followed by an intravenous injection the 4th week. Then the rabbits were bled weekly for 3 weeks. Immuno-diffusion tests of the serum were used as a qualitative probe for the presence of dsRNA in fungal extracts. A second series of injections were given 1 month after the last bleeding. These experiments have been discontinued because the attempts to produce antibodies to dsRNA were unsuccessful.

Double-stranded RNA from Protoplasts of *Endothia parasitica* (EP-49)

GILLIES, Kevin, VAN ALFEN, Neal, SIMMONS, John, and BOWMAN, James
Utah State University
Department of Biology
Logan, Utah 84322

Protoplasts were obtained from 3- to 4-day-old mycelium of EP-49, a European hypovirulent strain of *Endothia parasitica*, by glucuronidase-cellulose digestion of the cell walls in an isotonic solution. Double-stranded RNA (dsRNA) extracted from osmotically lysed protoplasts was compared with dsRNA from glass-bead-homogenized mycelium of EP-49 using polyacrylamide gel electrophoresis. The two dsRNA preparations exhibited the same gel pattern. These data indicate that glass-bead homogenization does not result in fragmentation of the dsRNA genome.

Although either method of cell disruption yields the same dsRNA species, glass-bead homogenization yields more dsRNA per gram of mycelium.

Attempts to Liberate Protoplasts Enzymatically from Virulent Cultures of *Endothia parasitica*

STOCKHAUSEN, Anne
West Virginia University
Department of Plant Sciences
Morgantown, West Virginia 26506

A method that may be used to study the transmission of hypovirulence in *Endothia parasitica* is to remove the fungal cell wall enzymatically and infect protoplasts with double-strand RNA (dsRNA) obtained from hypovirulent strains. The five enzymes that have been used, singly and in combination, to liberate protoplasts are: B-glucuronidase, chitinase, laminarinase, helicase, and a *Trichoderma viride* culture filtrate. Of these, only B-glucuronidase has been effective in releasing protoplasts from either 2-day-old mycelial plugs after a 5- to 6-hour incubation period or germinating spores after a 12- to 24-hour period. During incubation, mycelia or spores are shaken rapidly (150 rpm) at room temperature (24°C) in the enzyme solutions to which 0.5 m $MgSO_4$ and 0.05 m Na-maleate are added to maintain protoplast stability.

Latent Hypovirulence Phenomena in *Endothia parasitica*

VAN ALFEN, Neal, GILLIES, Kevin, SIMMONS, John, and BOWMAN, James
Utah State University
Department of Biology
Logan, Utah 84322

Hypovirulence has frequently been identified with white, poorly sporulating cultures grown on potato dextrose agar. Such cultures, when tested for virulence, are usually either low in virulence or avirulent. Colonies of this description can be obtained with high frequency (1 to 5 percent) when conidia of virulent *Endothia parasitica* isolates are irradiated with ultraviolet light. Such colonies may be the result of induction of the hypovirulent factor which has otherwise been latent within the fungal cell. Such white colonies, inducible at levels much greater than normal mutation rates, have been tested for double-strand RNA (dsRNA) content and virulence. They are all low in virulence and contain no detectable dsRNA. Tests are currently being done to determine whether they are truly hypovirulent by testing their ability to convert virulent isolates to hypovirulent ones by cytoplasmic transfer.

D. Miscellaneous

Alternative (non-hypovirulence) Control Methods

JAYNES, Richard A.
Connecticut Agricultural Experiment Station
P.O. Box 1106
New Haven, Connecticut 06504

American chestnut trees were maintained for 3 and 4 years with annual injections of methyl-2-benzimidazolecarbamate (MBC) solutions. Control trees were either killed or seriously infected by *Endothia parasitica*. New cankers arose on treated trees, and infections present at the time of inoculation were not eliminated, but they were held in check. Foliage was injured on injected trees. Bioassays demonstrated that fungitoxic material is first translocated to the crown and then redistributed downward to the bark of the trunk. Redistribution of fungitoxic materials within the tree to new twigs and leaves formed the year after injection also was observed, as well as distribution downward into the roots.

A Correlation Between the Presence of Hamamelitannin and Blight Susceptibility in American Chestnut

ELKINS, John R.
Concord College
Division of Natural Sciences
Athens, West Virginia 24712

Aqueous extracts of bark from the blight-susceptible American chestnut, European chestnut, and chinquapin were found to contain large quantities of hamamelitannin, a diester of gallic acid and hamamelose (2-C-hydroxymethyl-ribose). In contrast, aqueous extracts of bark from the blight-resistant Chinese chestnut and Japanese chestnut and blight-resistant roots of American chestnut were found to contain essentially no hamamelitannin. In addition, a prominent unknown peak is present in bark extracts of most samples of the blight-resistant Japanese and Chinese chestnuts and is absent from bark extracts of most of the samples of the blight-susceptible American chestnut. The unknown peak is also present in extracts from most of the samples of European chestnut, which is less susceptible than the American chestnut, and in several samples from large surviving American chestnuts. Thus there does appear to be a correlation between blight susceptibility and the presence of hamamelitannin. No chemical correlation with resistance in large surviving

American chestnut has been observed, though the presence of the unknown peak may signal the possibility of resistance.

Evaluation of Chestnut Test Plantings in the Eastern United States

BERRY, Frederick H.
Northeastern Forest Experiment Station
USDA Forest Service
Delaware, Ohio 43015

Both the U.S. Department of Agriculture and the Connecticut Agricultural Experiment Station once had programs aimed at breeding blight-resistant chestnut trees. To test the performance of these hybrid chestnuts under forest conditions and compare their growth with that of Chinese chestnut species, 15 chestnut test plots were established in 13 eastern and midwestern states between 1947 and 1955.

In 1978, every living chestnut tree on the 15 plots was examined and data recorded on growth, blight resistance, and tree form. Out of a total of 1,746 trees planted, 26 percent survived. Survival of Chinese chestnuts was 37 percent; Connecticut hybrids 26 percent; and USDA hybrids 12 percent. USDA hybrids averaged 6.3 inches in diameter at breast height (dbh) and 44.7 feet high; Chinese chestnuts averaged 6.1 inches dbh and 43.4 feet high; and Connecticut hybrids averaged 5.5 inches dbh and 38.1 feet high.

Blight susceptibility of the trees was rated on a scale of 1 (severely blighted) to 5 (no blight). The average rating for both the hybrids and the Chinese species was about 4 (light). Forest tree form was also rated on a scale of 1 (valueless) to 5 (excellent). Average rating for USDA hybrids was 3 (average); Chinese chestnuts 2.7 (between average and poor); and Connecticut hybrids 2.5 (between average and poor).

From a summary of the data from all 15 plots, the 50 most promising trees were selected. At the time of examination, these trees were blight-free and had good to excellent timber form. Their growth rates were above average. Ten were USDA hybrids; 15 were Connecticut hybrids; and 25 were Chinese chestnuts. The 25 hybrid trees represented 10 percent of the 250 surviving hybrids. These 50 trees offer a good source for further genetic studies toward the development of blight-resistant chestnuts.

Role of Double-strand RNA-based Hypovirulence in Recovery of Chestnut from Blight in Italy

ELLISTON, John E.
Connecticut Agricultural Experiment Station
P.O. Box 1106
New Haven, Connecticut 06504

The specific role that double-strand RNA (dsRNA) based hypovirulence has played in recovery from chestnut blight over the past 30 years is unclear, primarily because many other factors may have been involved. Impressions gained from a month of examining chestnut stands in many parts of Italy include the following: The present density of chestnut sprouts in many mountainous areas of Italy is high and the incidence of blight cankers, given the density of the host, is low. Of several hundred cankers examined, abnormal, more or less superficial cankers with split, flaking bark predominate; typical lethal blight cankers are less abundant and normal cankers containing perithecia are present but appear to be rare. No perithecia were found on abnormal cankers. Samples from normal cankers yielded morphologically normal strains of *Endothia parasitica*. Bark samples from abnormal cankers yielded strains that were predominantly white in culture, mixtures of white and normal strains, or normal strains. All white

strains tested contained detectable levels of dsRNA and white strains displayed a wide range of pathogenicities in dormant American chestnut in the laboratory. Nine vegetative compatibility groups have been found so far among the isolates from Italy.

The relative absence of the ascospore stage, reducing gene recombination and airborne inoculum; the apparently small number of vegetative compatibility groups, minimizing obstacles to transmission of dsRNA; and unknown effects of host species and environment may have contributed to recovery by permitting dsRNA-based hypovirulence to become established and well distributed within the *Endothia parasitica* population in Italy. Extensive studies would be required to determine the roles of individual components of this complex interaction between host, parasite, pathogen(s) of the parasite, and environment. Agents conferring hypovirulence in the Italian isolates appear to eliminate the capacity of the fungus to produce the sexual stage without eliminating the capacity of the fungus to survive. Since the abundance of the sexual stage in North America appears to be an important obstacle to control of blight here, these agents may be most useful for biocontrol in North America.

Hypovirulence Offers Hope, Not Facts

KUHLMAN, E. George
Southeastern Forest Experiment Station
Research Triangle Park, North Carolina
27709

A recent Science (209: 597, 1979) article on the use of laetrile to control cancer ended by saying "Clinical trials on the drug conducted by the government are unlikely to resolve anything (in people's minds), because in the absence of a cure what people want is hope, not facts." At times hope for a cure can cloud scientific minds. In the late 1950's, a miracle cure for white pine blister rust was widely acclaimed without any experimental verification. Finally, experiments demonstrated there was no curative effect due to the treatment and a million-dollar program was quietly scrapped.

With hypovirulence in *Endothia parasitica*, we have been offered a lot of hope but few facts to substantiate the premise that hypovirulence is the factor enabling European chestnut to survive in Italy and France. Grente has no published results to show that treatment with hypovirulent strains would increase survival of European chestnut over what would occur in check plots. Grente's hypothesis is further "supported" by subjective statements on an association of hypovirulent isolates with apparently healing cankers.

In a Darwinian world of the survival of the fittest, hypovirulence appears to be at the bottom of a steep hill. With a reported rate of spread of 1 to 2 meters per year, hypovirulent isolates must compete with virulent isolates that spread 10 to 20 miles per year in the eastern U.S. forests. This translates into a rate that is 16,000 times slower. If the rate is increased by a thousand-fold, the hypovirulent forms will still take 16 years to cover a mile. Besides the slow rate of spread, hypovirulent forms are burdened with a reduced capacity for sporulation and survival. Dr. George Hepting has asked if the meek can inherit the chestnut world.

In the absence of solid experimental data to show that hypovirulence is controlling chestnut blight, an alternative hypothesis is that hypovirulence is the result of a less favorable host-pathogen relationship. Under these circumstances a resistant host or an unfavorable environment or both

limit canker development. When this happens the pathogen is debilitated either by becoming infected with the hypovirulence factor or because the factor goes from a suppressed to an active state.

☆U.S. GOVERNMENT PRINTING OFFICE: 1981-703-011/20

Smith, H. Clay.
1981. U.S. Forest Service American Chestnut Cooperators' Meeting. Northeast. For. Exp. Stn., Broomall, PA. 20 p. (USDA For. Serv. Gen. Tech. Rep. NE-64)

On January 8 and 9, 1980, a 2-day meeting of U.S. Forest Service American chestnut cooperators was held at Pipestem State Park, Pipestem, West Virginia. A total of 43 talks were given at this meeting. All speakers provided short abstracts of their talks; these abstracts are presented here.

443.3 : 176.1 (*Castanea dentata*)

Headquarters of the Northeastern Forest Experiment Station are in Broomall, Pa. Field laboratories and research units are maintained at:

- Amherst, Massachusetts, in cooperation with the University of Massachusetts.
- Beltsville, Maryland.
- Berea, Kentucky, in cooperation with Berea College.
- Burlington, Vermont, in cooperation with the University of Vermont.
- Delaware, Ohio.
- Durham, New Hampshire, in cooperation with the University of New Hampshire.
- Hamden, Connecticut, in cooperation with Yale University.
- Kingston, Pennsylvania.
- Morgantown, West Virginia, in cooperation with West Virginia University, Morgantown.
- Orono, Maine, in cooperation with the University of Maine, Orono.
- Parsons, West Virginia.
- Princeton, West Virginia.
- Syracuse, New York, in cooperation with the State University of New York College of Environmental Sciences and Forestry at Syracuse University, Syracuse.
- University Park, Pennsylvania, in cooperation with the Pennsylvania State University.
- Warren, Pennsylvania.